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Research Article

EFFECT OF LONG-TERM ARSENIC EXPOSURE ON FEMALE ALBINO RATS WITH SPECIAL REFERENCE TO THE PROTECTIVE ROLE OF *SPIRULINA PLATENSIS*

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ABSTRACT: Arsenic toxicity has important health concern as it affects different animal species all over the world. This study was designed to evaluate the histological changes caused by chronic arsenic exposure on some body organs of female albino rats, and the protective role of *Spirulina platensis*. In this experiment, 20 female albino rats were used. The rats were divided into four groups; control group and three groups that received spirulina (Sp), sodium arsenate and sodium arsenate plus spirulina respectively, for 3 months by oral gavages. Arsenic treated group revealed decreased level of serum estradiol (E2) in comparison to control group, while this level was improved with spirulina administration. Serum malondialdehyde (MDA) level was significantly increased in arsenic treated group as compared with control group and co-treatment with spirulina reversed this level to nearly normal. Serum glutathione (GSH) activity significantly reduced in arsenic administered group in comparison with the control. On the other hand, spirulina co-treatment significantly improved serum GSH levels. Arsenic treatment significantly increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Spirulina administration significantly recovered the serum AST and ALT levels. Histopathological findings revealed different degenerative and inflammatory changes in uterus, liver, heart, lungs and brain of arsenic treated group. Histological alterations were markedly improved by co-treatment with spirulina. Chronic arsenic exposure causes different histological alterations in body organs, and these changes can be improved by co-treatment with *Spirulina platensis.*

Key words: Chronic Arsenic exposure, female rats, histopathology, *Spirulina platensis.*

INTRODUCTION

Arsenic (As) is presently one of the most predominant environmental pollutants. (Chowdhury *et al*. 2016). Arsenic is a toxic metalloid contaminant found in natural drinking water sources throughout the world (Ramsey *et al*. 2013a). Arsenic poisoning occurs naturally in both organic and inorganic forms in water, food, soil, dust, wood and other materials. Inorganic arsenic is more toxic than organic form. From a half of century past, arsenic has been used in medicine, cosmetics industry and agriculture. Arsenic can cause acute, sub-acute and chronic poisoning (Sayed *et al.* 2015).

Gastro-intestinal, cardiac, renal, bone marrow, central nervous system and hepatic damage may be noted at different stages of arsenic poisoning and also can cause many other toxic effects. Recently, a series of animal and human epidemiological studies have indicated an association between arsenic exposure and adverse reproductive and developmental outcomes (Noman *et al.* 2015).

Arsenic-mediated toxic effect on liver, spleen and kidney has been documented in a few studies (Chowdhury *et al.* 2016). Arsenic toxicity is one of the risk factors associated with cardiovascular diseases that include direct myocardial injury, cardiac arrhythmia, cardiomyopathy, ischemic heart disease and blood pressure (Ahangarpour *et al.* 2018). Arsenic has attracted worldwide interest because it shows substantial anticancer activity in individuals with acute promyelocytic leukemia. Unfortunately, the use of these drugs is associated with cardiotoxicity. This may involve multiple mechanisms including the generation of reactive oxygen species (ROS) in cardiomyocytes, oxidative DNA damage and arsenic accumulation (Zhang *et al.* 2013). Several studies have

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established the link between arsenic exposure and increased lung cancer risk; however, emerging data suggest an important role for arsenic in non-malignant lung disease. Epidemiological data have linked arsenic exposure via drinking water to chronic cough, chronic bronchitis, bronchiectasis and obstructive lung diseases (Ramsey *et al.* 2013b). Since arsenic can cross the blood brain barrier (BBB), it accumulates in the brain and causes neurobehavioral abnormalities (Goudarzia *et al.* 2018). Neurological effects of Arsenic may develop within a few hours after ingestion but are usually seen 2–8 weeks after exposure. However, the exact mechanism of action of As-induced neurotoxicity is poorly understood. Neurological disorders have been correlated with disorganization and changes in the expression levels of neuronal cytoskeleton proteins (Vahidnia *et al*. 2008).

The toxicity of arsenic is thought to be caused by the signals spawned due to its reaction with sulfhydryl groups of various enzymes and proteins. In this way, arsenic can activate potential intracellular signaling pathways which ultimately lead to arsenic-mediated adverse health effects (Chowdhury *et al*. 2016).Understanding the organ specific histological effect of arsenic is necessarily important to know the details mechanism of arsenic mediated toxicity in mammals (Noman *et al.* 2015).The dietary antioxidants (vitamin C, E and β-carotene) may reduce the arsenic burden in human by increasing its metabolism. Clinical study suggests that algae having very high concentration of micronutrients and vitamins may have beneficial effects in heavy metal poisoning. Spirulina is not only a whole food but it seems to be an ideal therapeutic supplement (Sayed *et al*. 2015). Spirulina has been recommended as a chemoprotective against arsenic-induced toxicity in humans. It seems reasonable to expect that administration of spirulina might provide a protective mechanism against arsenic-induced toxicity (Ghosh *et al.* 2014).

This study was performed to determine the adverse effect of chronic arsenic toxicity on different body organs and to assess the protective potential of *Spirulina platensis* in female albino rats.

MATERIALS AND METHODS

Chemicals

Sodium arsenate (Na_3AsO_4) was obtained from the Fisons Scientific Apparatus Ltd. UK in Egypt. Spirulina was obtained from Arab Academy for Science, Technology and Maritime Transportation, Alexandria, Egypt. Kits for biochemical analysis were purchased from Biodiagnostics, Egypt.

Table 1. Scoring of histopathological lesions in different organs of all experimental groups.

Organs	Lesions	Control	SP	AS	SP+AS
Uterus	Degeneration of endometrial lining epithelium	0	0	2	0
Liver	Necrosis of endometrial glands	0	0	2	1
	Mucosal inflammatory cells infiltration	0	0	2	0
	Dilatation of hepatic sinusoids	0	0	3	1
	Vacuolar degeneration of hepatocytes	0	0	2	1
	Portal fibroplasia with infiltration of mononuclear inflammatory cells	0	0	3	1
Heart	Hyperplasia of bile ducts lining epithelium	0	0	2	0
	Cytoplasmic vacuolization of the sarcoplasm of myocytes	0	0	2	1
Lungs	Zenker's necrosis of myocytes	0	0	2	0
	Infiltration of myocytes with mononuclear inflammatory cells	0	0	2	0
Brain	Interstitial pneumonia	0	0	3	1
	Bronchiolitis	0	0	2	0
	Reduction in granular layer of cerebellum	0	0	2	0
	Decreased density of Purkinje cells	0	0	1	0
	Neuronal degeneration	0	0	2	1
	Glial cells proliferation	0	0	1	0

The scoring system was designed as: score 0 = absence of lesions in all rats of the group (n= 5).
Score 1= <30%, score 2= <30% – 50%, score 3= >50%. SP: spirulina, AS: arsenic.

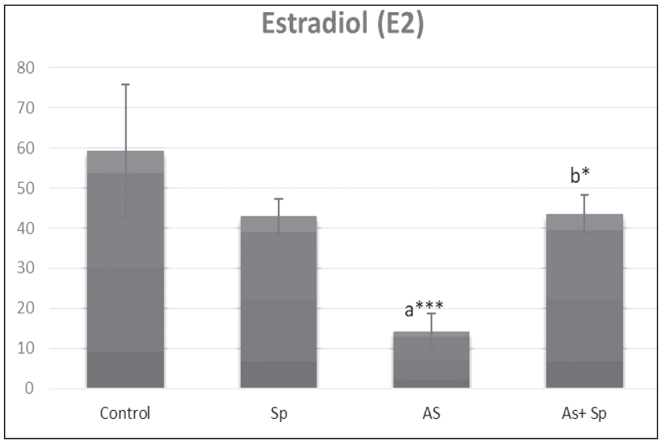

Fig. 1.Values of estradiol among different experimental groups (Mean ± SD).
[Superscript (a) refers to significance from control group, superscript (b) refers to a significance from As groups. * refers to p< 0.05, *** refers to p< 0.001. Sp: Spirulina; As: arsenic; As+Sp: Arsenic and Spirulina].

Experimental animals

Twenty adult female albino rats (weighing 120 - 150 gm) were used. Experimental rats were obtained from Helwan Animal Colony belonging to VACSERA. Rats were housed in standard cages kept in a ventilated room under controlled laboratory conditions of normal light-dark cycle (12 hours light/dark) and temperature (25 ± 2°C). Standard laboratory animal feed and water were provided *ad libitum*. Animals were kept to acclimatize for two weeks. The experimental protocol was approved by the Institutional Animal Care and Use Committee (CUIACUC), Cairo University, Egypt (approval No. CU-II-F-37-18).

Experimental Design

Rats were randomly divided into four groups (n = 5 rats). Group 1: rats received normal saline and acted as control group. Group 2: rats received daily Spirulina (Sp) (300 mg/ kg b.wt.) dissolved in water by oral gavage. Group 3: rats received daily sodium arsenate (5 mg/kg b.wt.) dissolved in normal saline orally and group 4: rats received daily Sp (300 mg/kg b.wt.) and sodium arsenate (5 mg/kg b.wt.) for 3 months (Bashandy *et al.* 2016).

Sampling

At the end of the experiment (after 3 months), rats were euthanized by decapitation and blood samples were collected from the retro-orbital venous plexus (Sorg and Buckner 1964) in sterile centrifuge tubes and left to clot and centrifuged at 3000 rpm for 15 minutes to separate sera. Sera aliquots were stored at - 20°C for further biochemical analysis. Uterus, liver, heart, lungs and brain from each rat were dissected and fixed in neutral buffered formalin 10% for histopathological examination.

Hormonal assay

Serum estradiol (E2) level was estimated using an enzyme-linked immunosorbent assay (ELISA) kit (Accu Bind, Monobind Inc, USA). 25 µL of each of the standards, control and treated serum samples were added to respective wells coated with anti-estradiol antibody and incubated with 200 µL of enzyme conjugate for 2 h at room temperature (RT). Subsequently, 100 µL of substrate was added and incubated for 15 min at RT. Reactions were stopped using 50 µL of stop solution and the optical density was measured at 450 nm with an ELISA reader (Multiskan, Thermo Lab Systems, Franklin, MA, USA).

Evaluation of lipid peroxidation and reduced glutathione

Lipid peroxidation was evaluated by measurement of serum MDA (Ohkawa *et al.* 1979) based on the formation of thiobarbituric acid reactive substances (TBARs) and expressed as the extent of malondialdehyde (MDA) production. The non-enzymatic antioxidant biomarker GSH was also assessed (Beutler *et al.* 1963).

Evaluation of liver function enzymes

Liver alanine aminotransferase (ALT or GPT) and aspartate aminotransferase (AST or GOT) activities were measured according to the method described by Reitman and Frankel (1957).

Histopathological examination

Uterus, liver, heart, lungs and brain were collected from the different experimental groups were routinely processed. The paraffin embedded blocks were sectioned at 5-micron thickness and stained with Hematoxylin and Eosin and Masson's Trichrome stain (MTC) (Bancroft *et al.* 2012) for histopathological examination by a light microscope (Olympus BX50, Japan).

Histopathological lesion scoring

Histopathological alterations in different organs (uterus, liver, heart, lungs and brain) were scored as, no changes (0), mild (1), moderate (2) and severe (3) changes, while the grading was determined by percentage as follows: < 30%changes were indicated as mild, < 30% – 50% indicated as moderate changes, and >50% indicated as severe changes (Arsad *et al.* 2014).

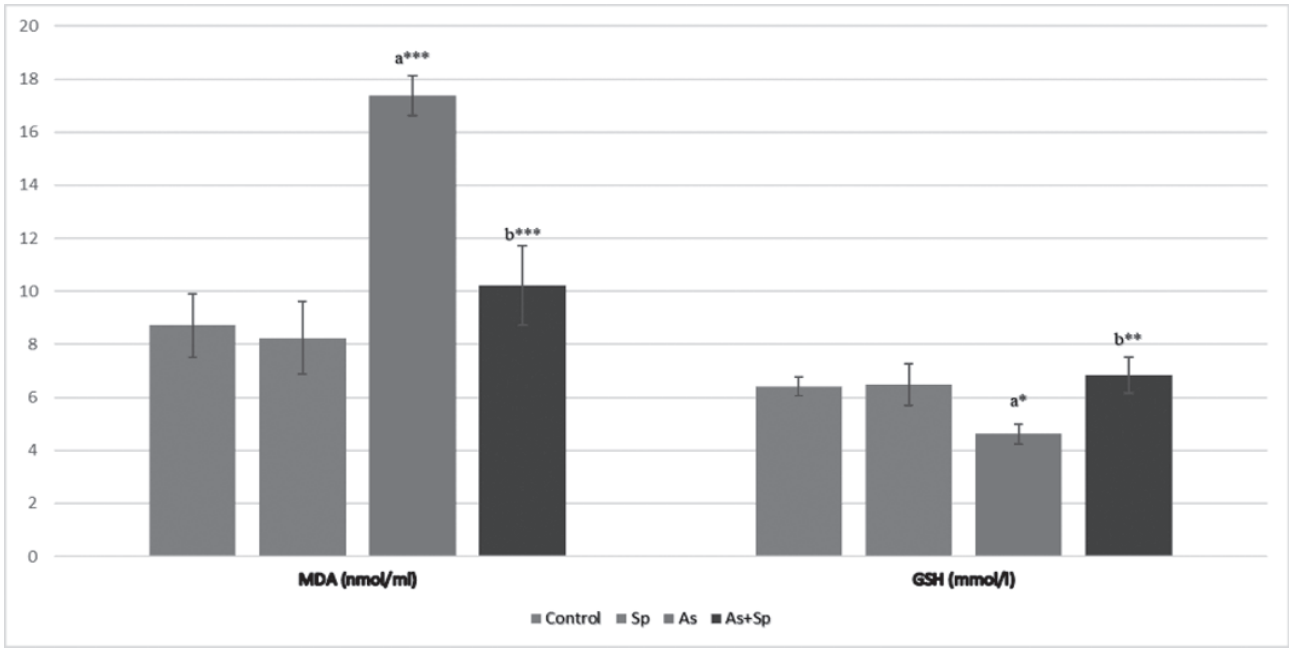

Fig. 2.Values of serum MDA and GSH among different experimental groups (Mean ± SD). [Superscript (a) refers to significance from control group, superscript (b) refers to a significance from As groups. * refers to p< 0.05, ** refers to p < 0.01, *** refers to p < 0.001. Sp: Spirulina; As: arsenic; As+Sp: Arsenic and Spirulina; MDA: Malonaldehyde; GSH: Reduced glutathione].

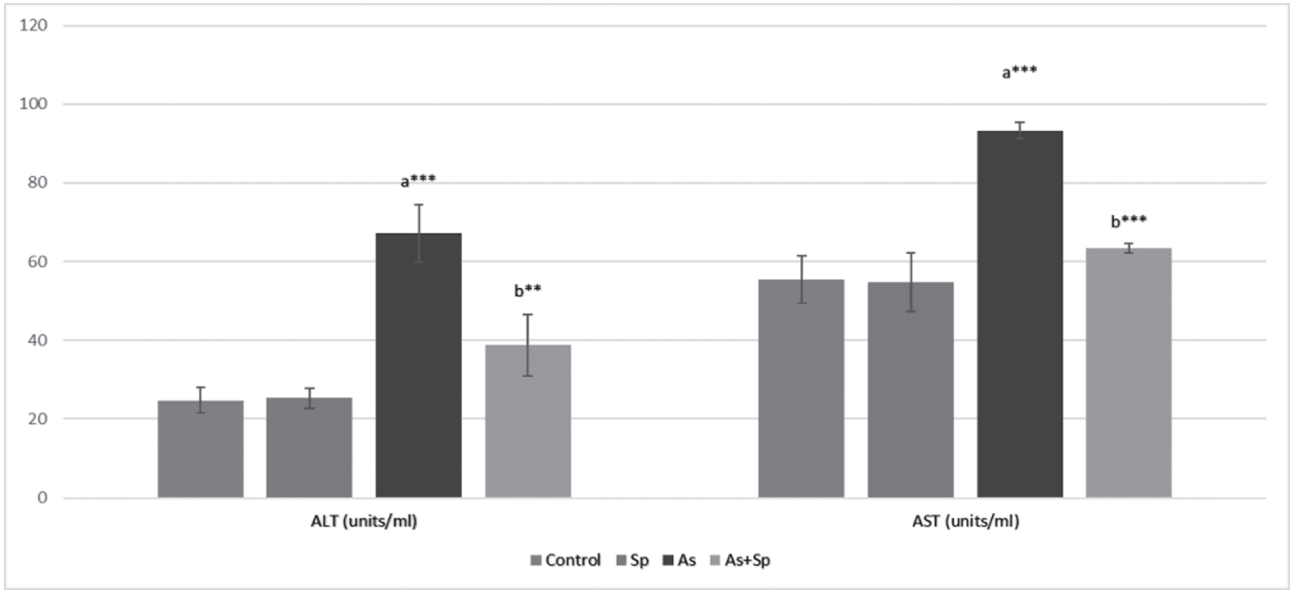

Fig. 3.Values of serum ALT and AST among different experimental groups (Mean ± SD).
[Superscript (a) refers to significance from control group, superscript (b) refers to significance from As groups. ** refers to p < 0.01; *** refers to p < 0.001. Sp: Spirulina; As: arsenic; As+Sp: Arsenic and Spirulina; ALT: alanine aminotransferase; AST: asprtate aminotransferase].

Statistical analysis

All results were expressed as mean ± SD (Standard Deviation). Statistical analyses were performed using the SPSS version 24.0 statistical analysis package (SPSS, Inc., Chicago, IL, USA). The parametric test one-way ANOVA was used for data analysis and comparison was done using Turkey post-hoc test. In all calculations, a difference at $p < 0.05$ or $p < 0.001$ was considered significant.

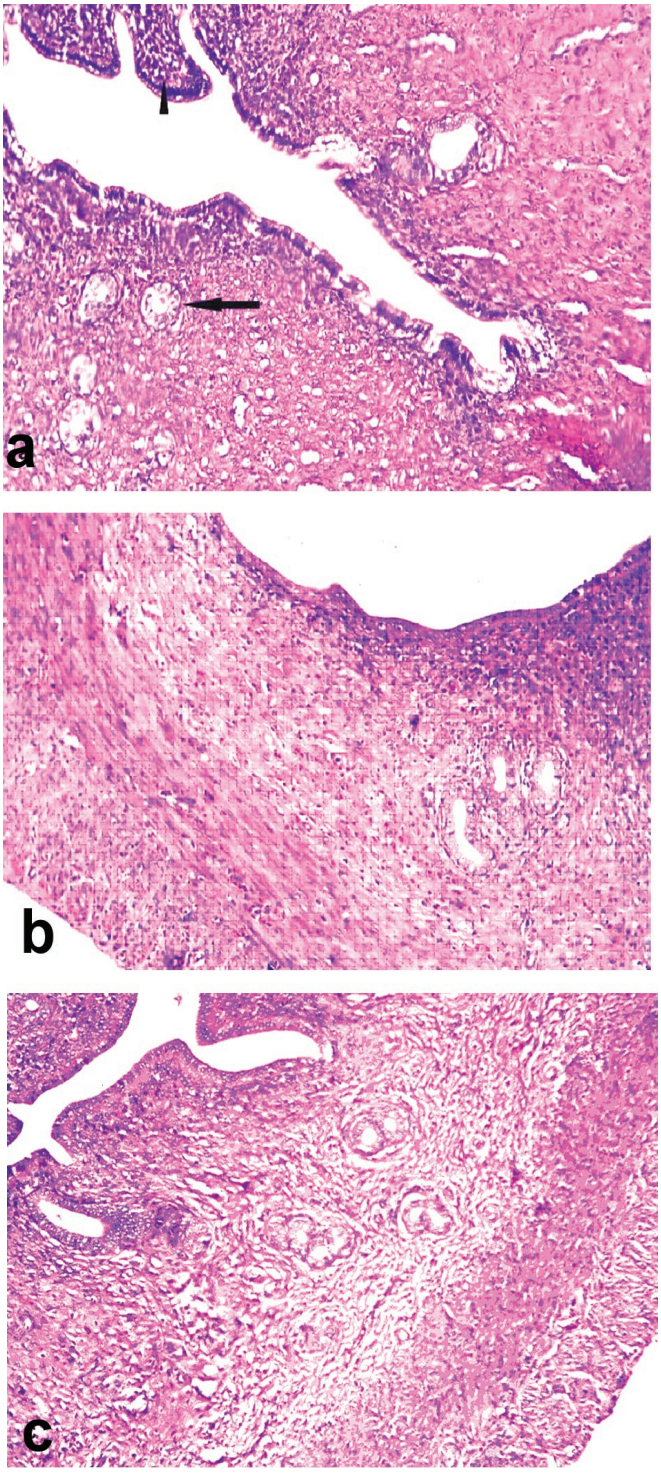

Fig. 4. Histological section of uterus of female rat.
[Control untreated group showing the normal histological structure of the uterus with normal endometrial lining epithelium (arrow head) and normal uterine glands (arrow) (a). Arsenic treated rat showing decreased invaginations of the uterine lumen with decreased endometrial glands number (b). Co-administration with spirulina showing noticeable increase in the number of uterine glands (c). (H&E X100)].

RESULTS AND DISCUSSION

Serum estradiol (E2) level

The different levels of E2 were presented in Fig.1. The levels showed significant decrease ($p < 0.001$) in As-treated group in comparison with control group. The E2 levels showed a significance ($p < 0.05$) recovery in spirulina co-treated group.

Serum malondialdehyde (MDA) and glutathione (GSH) levels

Serum MDA and GSH in different experimental groups were illustrated in Fig. 2. The results revealed significant elevation ($p < 0.001$) of serum MDA in As-treated rats in comparison with control group, and co-treatment with *Spirulina platensis* revealed a significant decrease of MDA levels in comparison with As-treated group ($p < 0.001$). A significant depletion ($p < 0.05$) of serum GSH in the As-treated rats was noted in comparison with control group, and co-administration with *Spirulina platensis* resulted in significantly increased ($p < 0.01$) level of serum enzyme GSH in comparison with As-treated rats.

Liver function enzymes

Levels of serum ALT and AST in different experimental groups were illustrated in Fig. 3. Serum ALT and AST levels exhibited significant increase ($p < 0.001$) in As-treated animals when compared with control group. These parameters showed a significant recovery ($p < 0.01$) when rats co-administered *Spirulina* with arsenic.

Histopathological findings

Uterus of control and spirulina treated rats revealed the normal histological organization and architecture with normal invagination of uterine lumen and normal uterine glands (Fig. 4a). On the other hand, uterus of arsenic treated rats showed degeneration of uterine endometrial lining epithelium and mucosal inflammatory cells infiltration. Endometrial glands also were affected as their number was decreased and some endometrial glands showed necrosis of their lining epithelium (Fig. 4b). These changes in the uterus were less obvious in group that co-administered with spirulina (Fig. 4c).

Microscopic picture of liver of control and spirulina treated groups, showed normal histological structure (Fig. 5a); while arsenic treated group showed marked activation of Kupffer cells, severe dilatation and congestion of hepatic sinusoids, sinusoidal leukocytosis and vacuolar degeneration of some hepatocytes while others showed

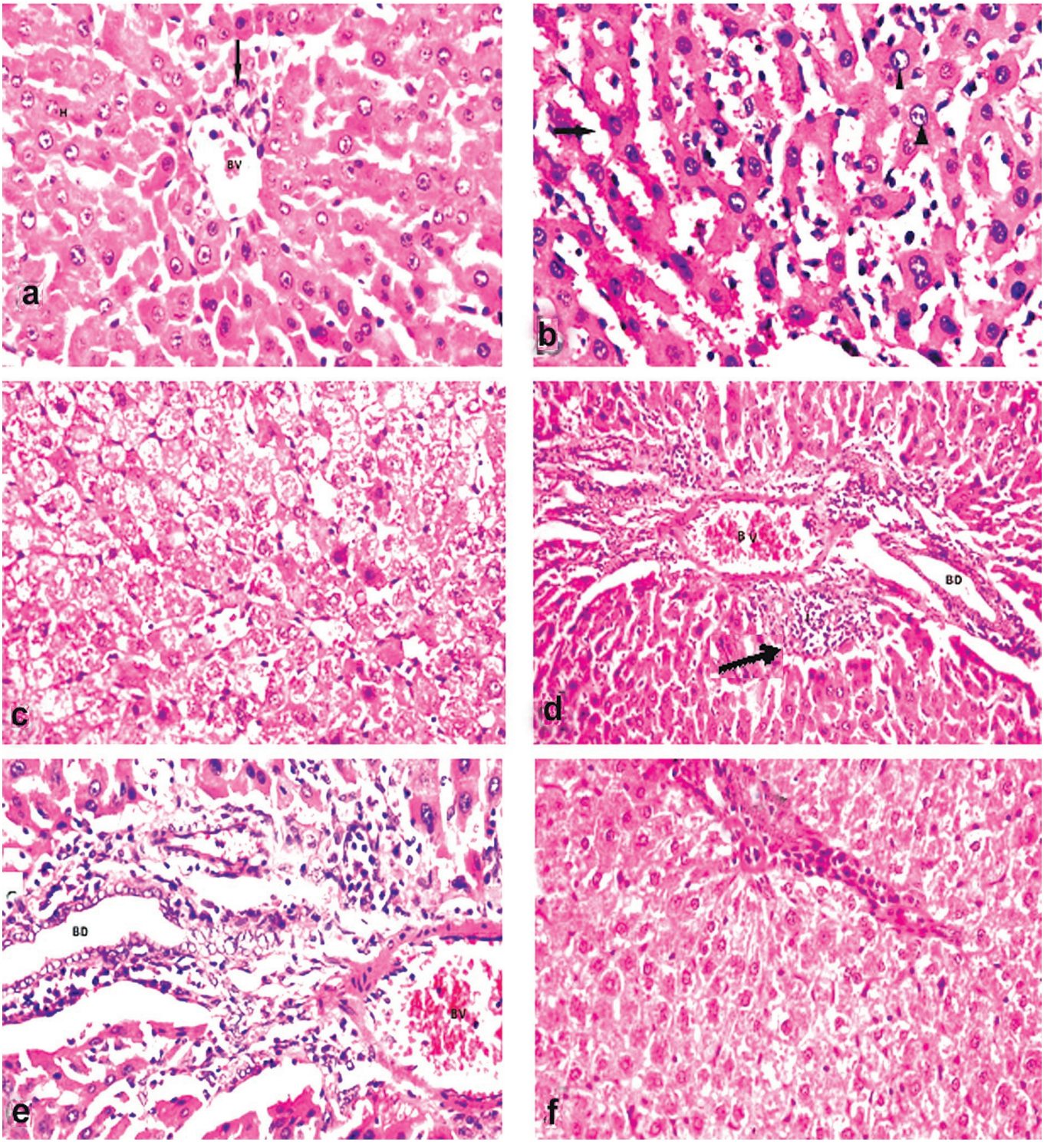

Fig. 5. Histological section of liver of female rat.

[Control untreated group showing the normal histological structure of hepatocytes (H), portal blood vessel (BV) and bile ducts (arrow) (a) (H&E X 400). Arsenic treated group showing severe sinusoidal dilatation (arrow), sinusoidal leukocytosis and karyomegaly (arrow head) (b); vacuolar degeneration of hepatocytes (c); portal area showing portal fibrosis with infiltration of mononuclear cells (arrow) and congestion of portal blood vessels (BV) (d) (H&E X 200); higher magnification showing hyperplasia of bile ducts (BD) (e) (H&E X 400). Spirulina co-treated group showing slight vacuolation of hepatocytes with slight congestion of portal blood vessels and few mononuclear cells infiltration in portal area (f) (H&E X 400)].

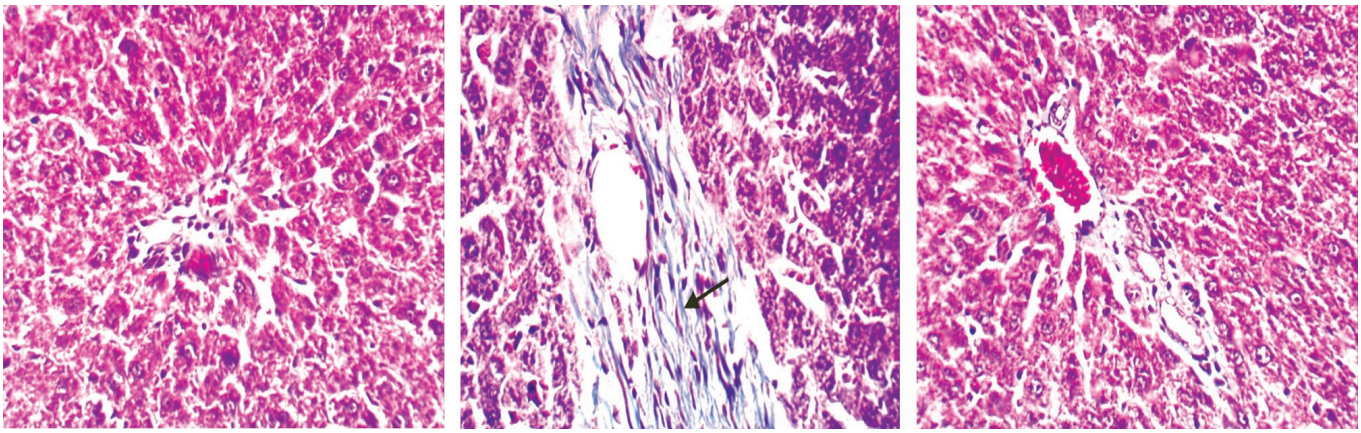

Fig. 6. MTC-stained liver sections.
[Control group showing no deposition of collagen in portal area (a) (MTCX400). Arsenic treated group showing extensive deposition of collagen in portal area which appearing blue in color (arrow) (b) (MTCX400). Spirulina co-treated group showing reduced collagen deposition in portal area (c) (MTCX400)].

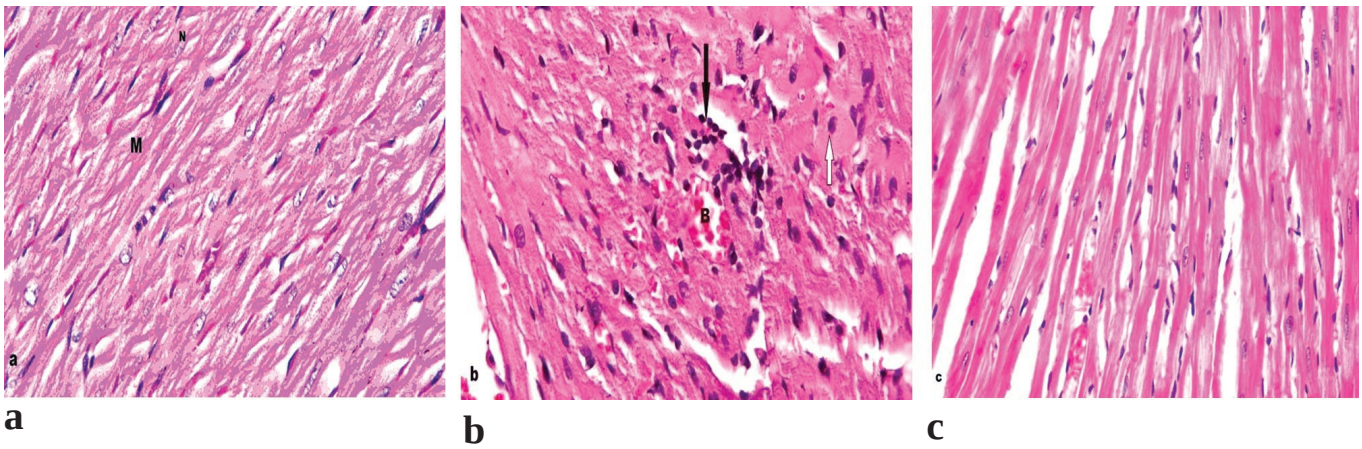

Fig. 7. Histological section of myocardium of female rat.
[Control untreated group showing the normal histological structure of myocytes (M) and their nuclei (N) (a) (H&E X600). Arsenic treated group showing myositis with infiltration of mononuclear cell (black arrow), Zenker's necrosis of focal myocytes (white arrow) and congestion of inter-myocardial blood vessels (B) (b) (H & E X600). Co-administration with spirulina showing normal structure of myocytes (c) (H&E X400)].

karyocytomegaly (Fig. 5b and 5c). Portal areas showed fibroplasia with infiltration of mononuclear inflammatory cells, congestion of portal blood vessels and hyperplasia of epithelial lining bile ducts with formation of newly formed bile ductules (Fig. 5d and 5e). Additionally, sporadic hepatocellular necrosis was noticed. These alterations were ameliorated by spirulina co-treatment as examined sections revealed slight vacuolation of hepatocytes with slight congestion of portal blood vessels and few mononuclear cells infiltration in the portal triad (Fig. 5f). Portal fibrosis and collagen deposition in portal areas of liver in different treated groups were confirmed by MTC. MTC stained liver sections showed extensive deposition of collagen in portal areas in arsenic treated group (Fig. 6b), and mild collagen deposition was noticed in spirulina co-treated group (Fig. 6c).

Histopathological examination of heart revealed normal histological structure of cardiac myocytes in both control and spirulina groups (Fig. 7a). Heart of rats intoxicated with arsenic showed cytoplasmic vacuolization of sarcoplasm of myocytes, focal zenker's necrosis of myocytes associated with infiltration of mononuclear inflammatory cells and congestion of inter myocardial blood vessels (Fig. 7b). These changes disappeared with co-administration of spirulina (Fig. 7c). Some examined sections from this group revealed slight focal cytoplasmic vacuolization of the sarcoplasm of myocytes.

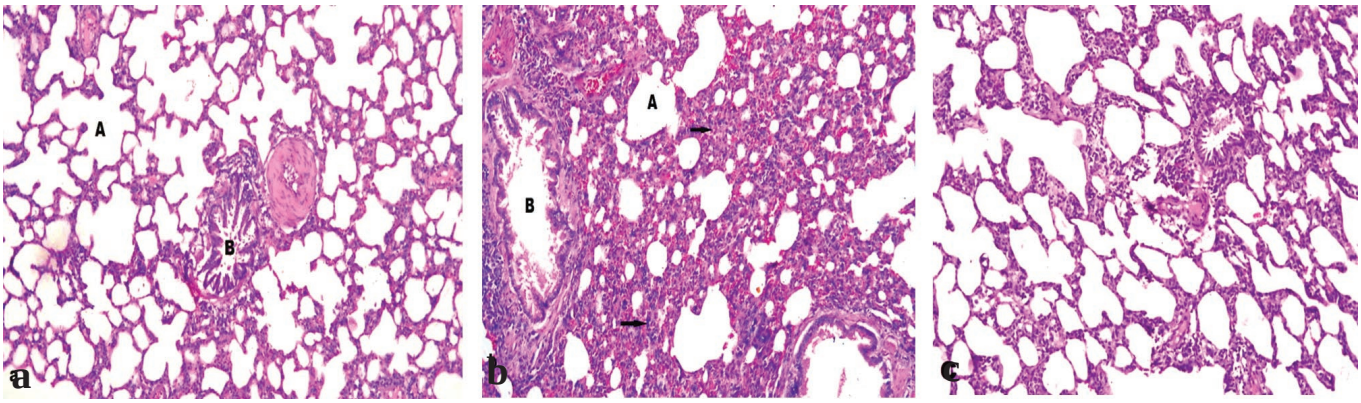

Fig. 8. Histological section of lungs of female rat.
[Control untreated group showing the normal histological structure of lung alveoli (A) and bronchiole (B) (a). Arsenic treated group showing thickening of alveolar wall by mononuclear inflammatory cells infiltration and dilated perialveolar blood capillaries (arrows), there is peribronchiolar mononuclear inflammatory cells infiltration (b). Co-administration with spirulina showing decreased thickness of alveolar wall with few numbers of mononuclear cells infiltration (c) (H&E X200)].

Lungs showed normal histological structure in both control and spirulina administered groups (Fig. 8a). Arsenic treated group revealed interstitial pneumonia in which there was thickening of alveolar wall, infiltration of mononuclear cells and congestion of perialveolar blood capillaries. Bronchioles showed bronchiolitis in which there was infiltration of mononuclear cells in the wall (Fig. 8b). Bronchiolar lumen contained exudate mixed with mononuclear cells with hyperplasia of bronchiolar lining epithelium. Co-administration with spirulina showed improved thickness of alveolar wall with few mononuclear cell's infiltration (Fig. 8c).

Microscopically, brain of control and spirulina treated rats showed the normal histology of both cerebellum and cerebrum (Fig. 9a and 9d). As-treated group showed reduction in granular layer (GL), decreased density of Purkinje cells (DPc) (Fig. 9b), neuronal degeneration with formation of neurofibrillary tangles in some degenerated neurons (Fig. 9e) and glial cells proliferation and astrocytic edema in cerebral cortex (Fig. 9f). Co-administration of spirulina demonstrated a dramatic improvement in the structure of brain tissue in both cerebellum and cerebrum (Figs. 9c and 9g).

Histopathological lesion scoring

All the recorded lesions in the uterus, liver, heart, lungs and brain were scored according to their severity as shown in Table 1.

Arsenic, one of the high-ranking global environmental toxicants, has currently drawn increasing interest as a major contaminant of food-chain and drinking water. Arsenic (As) is present in different forms in the environment and the toxicity relies upon its chemical forms and oxidation states (Al-Forkan *et al.* 2016). This study was performed to assess the effect of chronic arsenic toxicity on some body organs of female albino rats and possible protective effect of spirulina.

In our study, the level of serum estradiol showed significant decrease in As-treated group. These levels showed a significant recovery in spirulina co-treated group. Arsenic can alter steroidogenesis by its action as endocrine disruptor chemical (EDC). EDCs can alter the hypothalamic-pituitary-gonadal function, estrogen and androgen synthesis and their receptor mediated effects in mammals and other animals. Arsenic as potent EDC alters the gene regulation by closely related receptors of glucocorticoids, mineralocorticoids, progesterone, androgen and estrogens (Jahan *et al.* 2012). Very low doses of arsenic enhance the hormone mediated gene transcription, whereas higher doses act as suppressor to these hormones. So, the arsenic may alter some common target or process in the steroid receptor mechanism of action. All steroid receptors are affected by arsenic in a similar manner and showed a complex dose-dependent response to arsenic (Mondal *et al.* 2013).

There was a significant elevation of serum MDA in As-treated rats in comparison with control group, and co-treatment with *Spirulina platensis* revealed a decrease of MDA levels. Decreased serum GSH in As-treated rats was recorded, and co-administration with *Spirulina platensis* resulted in an increase in serum antioxidant capacity (GSH). Arsenic can cause elevation of MDA levels. Arsenic exposure enhances the lipid peroxidation and leads to an oxidative stress as a result of ROS production and lipid peroxidation of membranes, thereby causing degradation of phospholipids and finally cellular

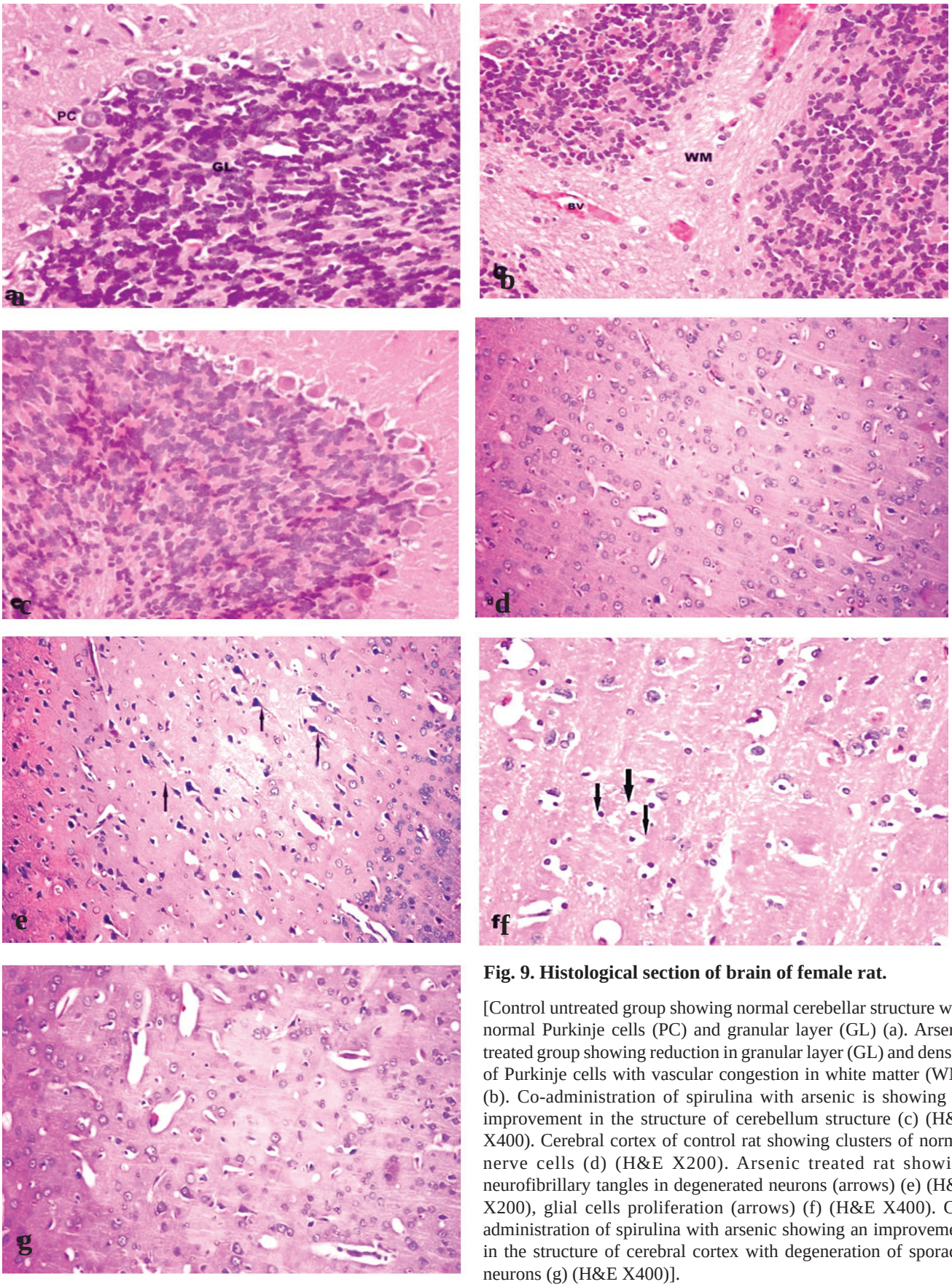

Fig. 9. Histological section of brain of female rat.

[Control untreated group showing normal cerebellar structure with normal Purkinje cells (PC) and granular layer (GL) (a). Arsenic treated group showing reduction in granular layer (GL) and density of Purkinje cells with vascular congestion in white matter (WM) (b). Co-administration of spirulina with arsenic is showing an improvement in the structure of cerebellum structure (c) (H&E X400). Cerebral cortex of control rat showing clusters of normal nerve cells (d) (H&E X200). Arsenic treated rat showing neurofibrillary tangles in degenerated neurons (arrows) (e) (H&E X200), glial cells proliferation (arrows) (f) (H&E X400). Co-administration of spirulina with arsenic showing an improvement in the structure of cerebral cortex with degeneration of sporadic neurons (g) (H&E X400)].

deterioration. Depletion of glutathione level after arsenic exposure may be because of enhanced ROS production and oxidative stress that can result in increased consumption of glutathione. Glutathione has been shown as an important biomarker of oxidative stress, which forms the first line of antioxidant defense against arsenic-induced damages. A reduction in tissue GSH level indicates oxidative damage (Ahangarpour *et al.* 2018).

Serum ALT and AST levels showed significant increase in As-treated animals when compared with control group. These parameters showed recovery when rats co-administered *Spirulina* with arsenic. This increase indicates cellular leakage and failure of functional integrity of liver cell membranes. Because of its unique metabolic functions, liver is an important target organ of toxicity. Oxidative stress is known to associate with this toxicity (Chowdhury *et al.* 2016).

In this study, uterus of the arsenic treated rats histologically showed degeneration of the uterine endometrial lining epithelium with decreased number of endometrial glands. Arsenic is an endocrine disruptor; influences sex hormones and induces inhibition of ovarian steroidogenesis and reproductive disturbances (Sun *et al.* 2016). Arsenic toxicity also causes inhibition in gonadotropin secretion (Chatterjee and Chatterji 2011). Uterine weight and histology are regulated by plasma estradiol level. Uterine endometrium degeneration is associated with increased production of reactive oxygen species (ROS). Endometrial stroma regulates the growth and function of endometrial glands. Possibly disrupted endometrial stroma may affect the growth and differentiation of endometrial glands (Korany *et al.* 2019).

Microscopic picture of liver of arsenic treated group showed severe changes and liver has been identified as a target organ of arsenic exposure. Because of its unique metabolic functions and related to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics. Like other toxic elements arsenic primarily increased the generation of free radical species and cause an imbalance between pro-oxidation and antioxidant homeostasis in liver system as a result causes hepatic degeneration. Arsenic mediated oxidative stress is associated with expression of antioxidant genes. Moreover, arsenic-induced pathological changes may be caused by oxidative DNA damage other than nitrative DNA damage. Oxidative stress through chronic arsenic exposure is associated with methyl insufficiency and loss of DNA methylation in animals may be reason for the histological changes (Noman *et al.* 2015).

In this work, histopathological findings of heart showed severe changes. Heart is one of the main target organs in arsenic toxicity (Ahangarpour *et al.* 2018). Several studies have indicated that cardio-vascular diseases are linked to the release of intracellular ROS which causes oxidative damages in heart and resulted in severe histological alterations under normal circumstances, cells can defend against ROS damage by means of endogenous oxidants, such as glutathione, vitamin C, and vitamin E, as well as with the involvement of various peroxidases in the cellular antioxidant systems. After exposure to arsenic, the antioxidant defense system cannot maintain the depletion (Zhang *et al.* 2013).

Histological findings of lungs in arsenic treated group showed interstitial pneumonia and bronchiolitis. In recent years, growing number of epidemiological reports have linked arsenic exposure via drinking water with a wide range of respiratory diseases. Controlled exposure studies in rodents have suggested that the lungs, perhaps due to its local arsenic metabolism and highly oxidizing microenvironment, may be uniquely susceptible to toxicity from orally ingested arsenic. Few studies have been suggested that oral arsenic modifies immune/ inflammatory responses in the lungs *in vivo*. Direct exposure of cultured macrophages with *in vitro* arsenic, or of airway macrophages via inhaled arsenic, suppresses antimicrobial and inflammatory functions. Arsenic increases alveolar-to-plasma permeability of the lung to bacteria, endogenous proteins (CC16), and exogenous small molecules suggests a novel mechanism by which orally ingested arsenic may interact with inhaled exposures to amplify lung injury and augment systemic penetration of airborne agents (Henderson *et al.* 2017).

Histopathological changes in brain of As-treated group might be due to oxidative stress which has been suggested by several investigators. It has been shown that arsenic generates free radical species including hydroxyl radicals, superoxide anions, dimethyl arsenic peroxy radical, dimethyl arsenic radical, nitric oxide (NO) and others and thus impairs the antioxidant system in the brain and other biological tissues. NO readily combines with DA and produces peroxynitrite anions and semiquinones, reactive species implicated to damage the biological membranes. Increased generation of peroxynitrite anions is also imminent as NO may readily combine with superoxide anion. NO in the central nervous system plays a multifaceted role as a neuromodulator and is also involved in brain development via regulation of synaptic plasticity besides regulating cerebral blood flow (Rajesh *et al.* 2010). Depletion of endogenous antioxidants resulted in destabilization of cellular lipid substances and induction of oxidative damage in brain as evidenced by

enhanced lipid peroxidation level. Elevated MDA levels also caused oxidative injury in rats treated with arsenic (Adedara *et al.* 2017).

All these histological alterations were less obvious in group that co-treated with spirulina. Spirulina was effective in lowering arsenic level from the arsenic loaded tissues in rats. The exact cause of this protective role in recovering tissue damages is not fully understood. However, it is known that spirulina is an enriched source of nutrients like protein, amino acid, iron, β-carotene, phycocyanin, γ-lenolenic acid, vitamin B1, B2, B3, B6, B12 and essential fatty acids which are very much helpful to maintain the normal health (Ahmed *et al.* 2019).

CONCLUSION

Chronic arsenic toxicity causes different histological alterations in body tissues. These changes can be ameliorated by *Spirulina platensis* co- treatment.

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